Improving the quality and nutrient content of palm kernel cake through fermentation with *Bacillus subtilis*

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Abstract

Palm kernel cake (PKC) is a waste of the palm oil industry. Indonesia as the largest palm oil producer in the world, produced 45-46% PKC. PKC can potentially be used as animal ration but its utilization for poultry is limited. Thus, the fermentation process was carried out to improve the utilization of PKC in poultry ration. An experiment was conducted to study the effect between doses of *Bacillus subtilis* inoculums and fermentation time to increase the quality and nutrient content of fermented Palm Kernel Cake (FPKC). Materials used in this study are 1) PKC obtained from Palm Kernel Processing Manufacture of Andalas Agro-Industry in Pasaman, West Sumatra. 2) *Bacillus subtilis* obtained from The Research Center of Applied Chemistry LIPI, Bogor. 3) Nutrient agar medium (NA) produced by Difoo - Becton Dickinson. 4) Rice bran 5) Aquades and mineral standard. The experimental design used is a completely randomized design (CRD) with 3 x 3 factorial and 3 replications. The first factor was doses of inoculum Bacillus subtilis: 3%, 5%, and 7%, respectively. The second factor was fermentation times: 2 day, 4 days, and 6 days. The parameters measured were the content of crude protein (CP) and crude fiber (CF), nitrogen retention, and digestibility of crude fiber of FPKC. The result of the study showed that there was significant interaction (P,0.01) between factor A and factor B. Each of factor A and factor B also showed a significant effect (P,0.01) on crude protein, crude fiber, nitrogen retention, and crude fiber digestibility. From this study, it can be concluded that fermented PKC with7% doses of *Bacillus subtilis* and 6 days fermentation time provides the best result as seen from 24.65% CP content, 17.35% CF content, 68.47% nitrogen retention, 53.25% CF digestibility of FPKC.

Keywords: *Bacillus subtilis, fermentation product, inconventional feedstuffs, inoculum dose, nutrient quality, palm kernel cake*

Introduction

Feed is one of the most important factors in poultry farming. One of the biggest components of production costs is the cost of feed that can reach up to 70%. If the cost of production can be reduced, greater profit will be made. One way to reduce the cost of this ration is by utilizing plantation waste such as palm kernel cake (PKC). As the largest palm oil producer country in the world, Indonesia produced 45 million tons per year of palm oil (Directorate

General of Plantation, 2016).

PKC is one of the palm oil industry wastes that can be used as poultry feed. PKC is a by-product of extraction of palm kernel obtained through chemical and mechanical processes that have the potential to be used as poultry feed. The nutritional content (%) of PKC was 87.3 DM, 16.1 CP, 21.3 CF, 8.2 Crude fat, 0.27 Ca, 0.94% P (Mirnawati et al 2010). Although the CP content of PKC is relatively high, its utilization remains in poultry rations. PKC can only be given to the level of 10% in broiler rations due to high CF content (Rizal, 2000). PKC has quite high crude fiber content but low in quality (Odunsei et al 2002; Ezhieshi and Olomu, 2004), therefore, the utilization of PKC is not maximal if given directly without any processing.

The limited use of PKC is also due to the high content of mannan in PKC. In accordance with the opinion of Daud et al (1993) that 56.40% of PKC consists of β-mannan. The high content of β-mannan in PKC is one of the limiting factors in the use of PKC in the ration because no mannan-breakdown enzyme is produced in the poultry digestive tract. Fermentation can change feed ingredients, that contain protein, fat and carbohydrates that are difficult to digest, to be easily digested. In addition, fermentation also adds good taste, aroma and improves the nutrient content and quality of feed ingredients (Saono1976; Mirnawati et al 2012 and Mirnawati et al 2013).

Fermentation with PKC is carried out using mannanolytic microorganisms or microorganisms that produce *Aspergillus niger*, *Eupennicillium javanicum*, and *Sclerotium rolfsii*. Mirnawati et al (2017) have studied the PKC fermentation with three mannanolytic fungi that produce mannanase enzyme (*Aspergillus niger*, *Eupenicilum javanicum* and *Sclerotium rolfsii*. From these three fungi, *Sclerotium rolfsii*. showed the best results as seen fromthe nutrient content, which was 27.43% crude protein, 11.53% crude fiber, 0.22% crude fat, 0.75% Ca, 0.85% P, 59.17% nitrogen retention, and 55.40% digestibility of crude fiber. This results had been applied in broiler ration up to 25% (Mirnawati et al 2018).

Besides fungus, there are also mannanolytic bacteria such as *Bacillus subtilis*. Pangsri and Prapawan (2017) found that the activity of the mannanase enzyme from *Bacillus subtilis* on tea waste was 0.80 U/ml higher than the coffee ground and locust bean gum, consecutively 0.68 and 0.15 U/ml. Tea waste and coffee grounds were suitable substrates for enzyme production. Besides that *Bacillus subtilis* can also be functioned as a probiotic. *Bacillus subtilis* could be detected in the small intestine of mice after they had received an oral doses of spores (Casula et al 2002; Tam et al 2016; Stephen et al 2008).

In the fermentation process, there are several factors that must be considered, including the doses of inoculum and the length of fermentation. The higher doses of inoculum and the longer fermentation time will results in The more doses of inoculum given, the more microbes that grow, while the longer the fermentation time is given, the more microbes can grow, so that the combination of inoculum doses and length of fermentation will be able to increase the quality and nutrient content of FPKC. A study to determine the doses of *Bacillus subtilis* as inoculum and the fermentation time to increase the quality and nutritional content of PKC became a necessary.

Methodology

The materials that were used in this experiment were: 1) Palm kernel cake derived from PT. Incasi Raya, 2) bacteria *Bacillus subtilis* obtained from The Research Center of Applied Chemistry LIPI Bogor, 3) medium (NA/ Nutrient Agar), 4) Aquades, 5) Buffer liquid with pH 7, 6) NaOH, 7) H_2SO_4, 8) acetone, 9) 21 Broiler chickens with the age of 4 weeks old, weighed ±1500 gram.

The experiment was conducted using the experimental method. The experiment was designed using completely randomized design (CRD) with 3 x 3 factorial arrangement of treatments with three replications. Factor A was doses of inoculums *Bacillus subtilis* which were as follows: (A1) 3%, (A2) 5% and (A3) 7%. Factor B was time fermentation which was as follows: (B1) 2 day, (B2) 4 day, (B3) 6 day. Data collection: nutrient content and quality of FPKC: crude protein (CP), crude fiber (CF), nitrogen retention and digestibility of FPKC. The collected data were subjected to statistical analysis Steel and Torrie (1991). Duncan's Multiple Range Test (DMRT) was applied to compare the difference between treatments.

The variable of crude protein and crude fiber were measured using proximate analyses, while nitrogen retention was measured by the Sibbald method (1976). This experiment used 6 weeks-old of 30 broiler chickens (27 for treatment and 3 for endogenous). Before the experiment, all chickens were fasted for 36 hours to avoid the influence of previous feeds. Each treated chicken is force-feeding with fermented products as much as 20 grams per head, and then the chicken is put into a metabolic cage that has been equipped with a drinking place and feces storage mechanism. Feces is collected every hour for total of 30 hours and every hour and sprayed with 0.3 N H_2SO_4 to avoid nitrogen evaporation. The collected feces is dried in an oven at a temperature of 50-60°C, ground until it become smooth, and then analyzed for nitrogen retention and crude fiber content of the faecal.

Results and Discussion

The effects of treatment on crude protein, crude fiber, nitrogen retention and digestion of crude fiber of FPKC are shown in Table 1.

Table 1. Mean content of crude protein, crude fiber, nitrogen retention and digestibility of crude fiber of FPKC

Parameters	Factor A (Inoculum dose)	Factor B (Fermentation time)			SEM	*p*
		B1 (2 days)	B2 (4 days)	B3 (6 days)		
Crude protein, % in DM	A1 (3%)	16.23^{bC}	16.66^{aC}	17.02^{aC}	0.18	0,01
	A2 (5%)	17.02^{cB}	18.71^{bB}	19.78^{aB}		
	A3 (7%)	19.16^{cA}	21.71^{bA}	24.65^{aA}		
Crude fiber , % in DM	A1 (3%)	26.89^{aA}	23.72^{bA}	19.25^{cA}	0.07	0,01
	A2 (5%)	26.30^{aB}	22.52^{bB}	18.69^{cB}		
	A3 (7%)	25.61^{aC}	21.38^{bC}	17.35^{cC}		
N retention, % N intake	A1 (3%)	50.32^{cC}	57.77^{bC}	63.92^{aB}	0.41	0,01
	A2 (5%)	53.38^{cB}	62.03^{bB}	64.79^{aB}		
	A3 (7%)	62.32^{cA}	65.04^{bA}	68.47^{aA}		

Crude fiber digestibility, %	A1 (3%)	45.32^{cC}	47.14^{bC}	51.36^{aC}	0.10	0,01
	A2 (5%)	45.85^{cB}	49.70^{bB}	52.27^{aB}		
	A3 (7%)	46.44^{cA}	50.91^{bA}	53.25^{aA}		

Difference superscript low-case letters in the same column and upper-case letters in the same line indicate a significant difference (P<0.01)

Effects of treatment on crude protein

The interaction between the dose of inoculum (factor A) and fermentation time (factor B) on crude protein content was significant, furthermore, the effect of factor A and factor B on crude protein content of PKC fermentation with *Bacillus subtilis* also highly significant. Based on table 1, there is an increase in crude protein content of each treatment along with the addition of more inoculum, both at dose 3, 5 and 7%. The longer fermentation time also increases the CP content of FPKC both on day 2, 4 and day 6. The highest crude protein is found in A3B3 treatment which is 24.6%. The high crude protein content in A3B3 treatment of fermented palm kernel cake with *Bacillus subtilis* is due to the more doses of inoculum given and the longer fermentation time. The A3B3 treatment causes more microbes to grow and multiply, while increased microbial growth contributes to high protein from the microbial body. According to Iyayi et al (2004) and Mathot et al (1992) that the increase in protein was thought to be due to the addition of proteins donated by microbial cells due to its growth, which produced a single cell protein product (SCP) or cell biomass containing about 40-65% protein. Details of the treatment effect can be seen in figure 1.

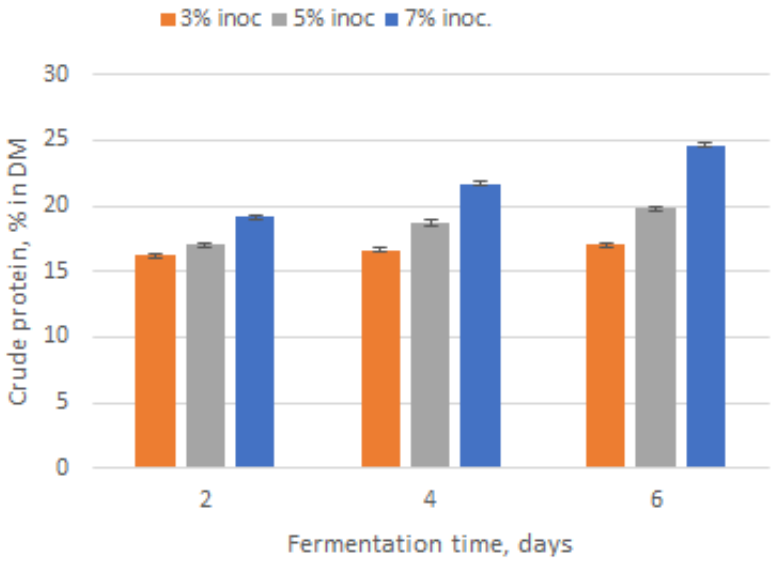

Figure 1. Fermentation of palm kernel cake with *B. subtilis*. Effect of concentration of the inoculum and the duration of the fermentation on the percent crude protein

Increased crude protein is also caused by the presence of enzymes produced by microbes. The more increasing number of microbes in the fermentation process, the more enzymes, which are proteins, will be produced (Mirnawati et al 2010). Thus, we can conclude that the presence of enzymes will affect crude protein content in feed ingredients. Mirnawati et al (2012) also found that during the fermentation process microbes will produced enzymes, which are proteins, while the microbes themselves are sources of single cell proteins. Thus

at the end of fermentation, there will be an increase of protein from fermented products (Mirnawati et al 2013).

Effect of treatment on crude fiber

The results showed that there was a highly significant interaction (P,0.01) between factor A (doses of inoculum) and factor B (fermentation time). Furthermore, factor A and factor B also had a highly significant effect on crude fiber content of FPKC.

The more doses of the inoculum given, the more the crude fiber content decreased. The longer fermentation time also decreases the crude fiber content of FPKC both on day 2, 4 and 6. Provision of inoculum doses and the appropriate length of fermentation time will provide an opportunity for bacteria to grow and develop properly so that more ingredients will be overhauled and more enzymes are produced, especially cellulolytic to grow the more these enzymes. According to the opinion of Sudarmono et al (2016) that the more fungi grow the more the cellulose produce to break down cellulose, so at the end of fermentation the crude fiber content decreased. Details of the crude fiber can be seen in figure 2.

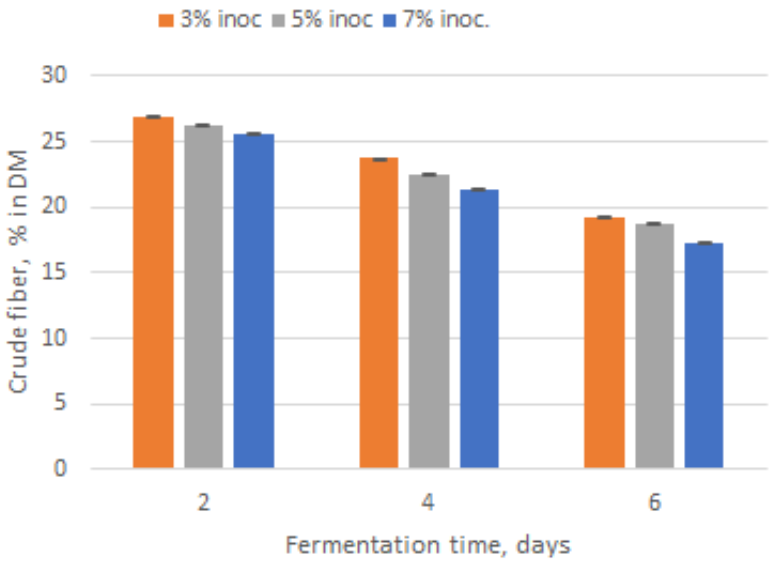

Figure 2. Fermentation of palm kernel cake with *B. subtilis*. Effect of concentration of the inoculum and the duration of the fermentation on the percent crude fiber

As seen from figure 2, the low crude fiber content in the A3B3 treatment was the effect of the high cellulose activity in this treatment. This cellulose enzyme will degrade cellulose to glucose so that at the end of fermentation the crude fiber content decreases. According to Ofuya and Nwajiuba (1990) that the more microbes that grow, the more cellulase produced to remodel cellulose into glucose, resulting in a decreased content of crude fiber at the end of fermentation. (Mirnawati et al (2013); Rizal et al (2013); Mirnawati et al (2017) found that there was a decrease of crude fiber in PKC after fermentation.

Effect of treatment on nitrogen retention

There was an interaction between the inoculum dose (A) and fermentation time (B) on nitrogen retention (Table 1), so each factor led to an increase in nitrogen retention after

fermentation with *Bacillus subtilis* (Figure 3).

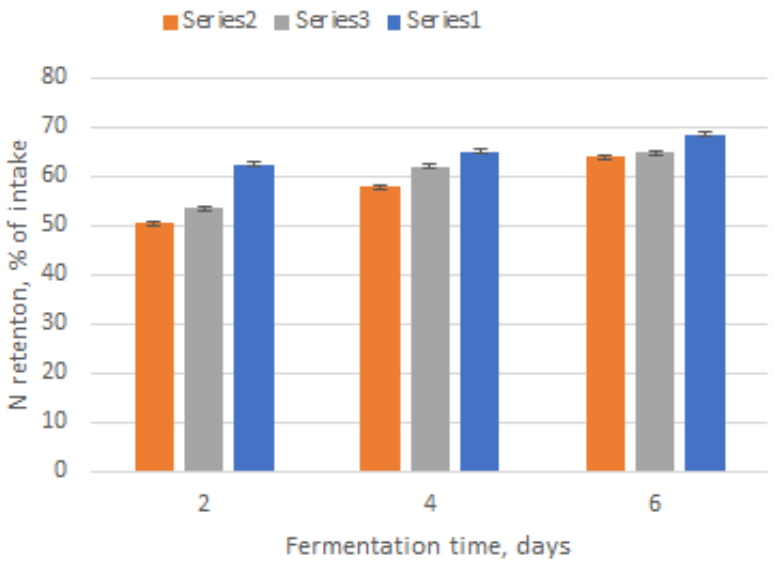

Figure 3. Fermentation of palm kernel cake with *B. subtilis*. Effect of concentration of the inoculum and the duration of the fermentation on N retention by broiler chicken

The increased in nitrogen retention in the treatment A3B3 is due to the higher crude protein content consumed than released through feces and urine. According to Mirnawati et al (2017) who found that nitrogen retention will be positive if more nitrogen is consumed compared to being excreted through feces and urine. The increase in retention of nitrogen is also due to better nutrient quality and more amino acids completed after the fermentation process. The fermentation process can change the complex components into simple one and improve nutrient quality so that the protein content in the substrate is transformed into better quality of amino acids and easily digested by livestock. Furthermore, high nitrogen retention in A3B3 treatment is also because the enzymes produced by the bacteria can change the structure of proteins into simple one Mahfudz et al (2004).

Effect of treatment on digestibility of crude fiber

There was a highly significant interaction (P,0.01) between factor A (dose of inoculum) and B (fermentation time), while each factor A and B also had highly significant effects (P,0.01) on crude fiber digestibility (Table 1). Increasing inoculum doses from 3, 5 to 7% showed an increase in digestibility of crude fiber of FPKC. Moreover, the increase in fermentation time will also increase the digestibility of crude fiber of KC on day 2, 4 and 6 respectively. Proper inoculum doses and fermentation time will give the bacteria the opportunity to grow and develop well so that more ingredients will be available and good fermented products will be produced. The highest digestibility of crude fiber is found in the A3B3 treatment (7% inoculum dose and 6 days fermentation time). Details of crude fiber digestibility in the treatment are shown in figure 4.

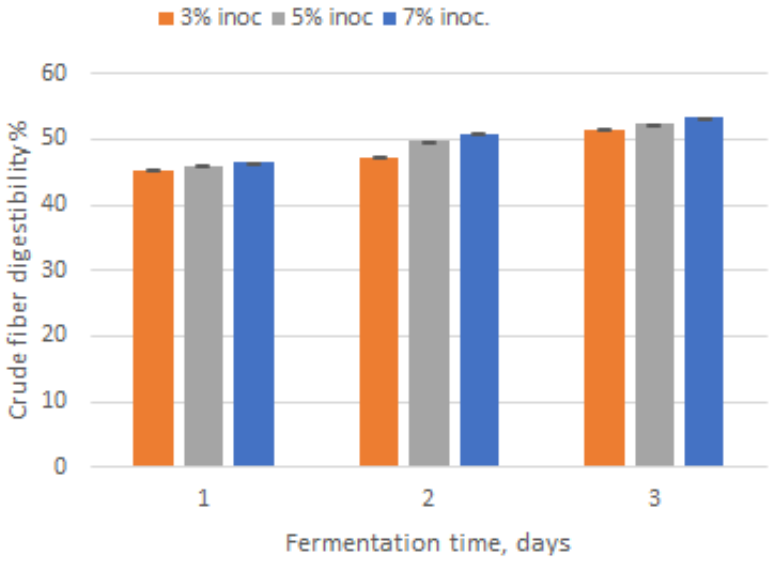

Figure 4. Fermentation of palm kernel cake with *B. subtilis*. Effect of concentration of the inoculum and the duration of the fermentation on crude fiber digestibility by broiler chicken

The high digestibility of crude fiber in the A3B3 treatment is related to the low crude fiber content available so that a lot of content is stored and utilized properly and increases the crude fiber digestibility of PKC fermented. This is supported by the opinion of Mirnawati et al (2017) that the digestibility of crude fiber depends on the crude fiber content in the feed ingredients; the higher the crude fiber content, the lower the digestibility of crude fiber due to the limitations of poultry to digest crude fiber. Digestion is also influenced by several factors including crude fiber content in the feed, composition of crude fiber preparation, and microorganism activity (Maynard et al 2005). Kassim et al (1985) found that the more cellulose produced to break down cellulose into glucose will result in an increased digestibility of crude fiber. The duration of fermentation also affects the digestibility of crude fiber, where fermented food substances usually have better nutritional value than the original ingredient because catabolic microorganisms will breakdown complex components into more simple ones, thus they become easier to digest (Walugembe et al 2014).

Conclusion

- Palm kernel cake which was fermented with *Bacillus subtilis* showed that inoculum dose of 7% and 6 days fermentation time provide better nutritional content.
- This can be seen from 24.6% crude protein, 17.3% crude fiber, 68.5% nitrogen retention, and 53.3% crude fiber digestibility of FPKC.

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